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Human ES- and Induced Pluripotent Stem-Derived Cardiomyocytes. A Comparative Electrophysiological Study

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Special attention is directed to the potential application of human induced pluripotent stem (iPS) cell-derived cardiomyocytes for cardiac safety pharmacology and toxicology with fewer legal and ethical issues. Supply of commercially available products enables many researchers to test the utility in their own systems. Although variations of electrophysiological properties have been reported among pluripotent cell lines by classifying cardiac subtypes into nodal, atrial and ventricular cells, most cells are spontaneously contracting, which makes difficult to be implicated in adult human hearts. Thus, we sought objective description on action potential (AP) parameters recorded from perforated patch-clamped iCell cardiomyocytes (iCell-CMs, AJ/CDI) by systematically comparing with those from human ES-derived cardiomyocytes (hES-CMs, Cellartis). Mean +/- SE values of APD_{50} in iCell-CMs (382 +/- 38 ms, n=36) were significantly longer than those in hES-CMs (278 +/- 28 ms n=64), while APD_{90} in iCell-CMs (1210 +/- 346 ms, n=36) tended to be longer than those in hES-CMs (776 +/- 136 ms n=64). As for APD_{90}, despite of the large sample size, there was no statistical significance between iPS-CM and hES-CMs. Detailed analysis of the variation by Gaussian fitting revealed that marked differences in shapes of probability distribution between iPS-CMs and hES-CMs. Although further studies are necessary to know if the variation of AP parameters affect drug responses, our data provide important information for cardiac safety assessments.

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Examining the Causes and Consequences of Calcium Overload in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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Intracellular calcium overload has been linked to arrhythmias in conditions such as ischemia reperfusion, heart failure, catecholaminergic polymorphic ventricular tachycardia and digitalis intoxication. While this link has been extensively studied at the cellular level using animal models, there is a paucity of information on the causes and consequences of Ca^{2+} overload in healthy human myocytes. With the advent of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM), human myocytes are becoming more readily available. However, it remains unclear if these myocytes faithfully recapitulate all aspects of adult cardiomyocyte physiology. In order to determine if iPSC-CM will be a useful platform to study the causes and consequences of Ca^{2+} overload, we developed methodology to examine Ca^{2+} overload in beating clusters of iPSC-CM and in isolated myocytes. Spontaneous and field stimulated action potentials were measured with high resistance microelectrodes in spontaneously beating clusters of iPSC-CM while measuring cluster contraction with simultaneous video edge detection. Free intracellular Ca^{2+} was measured with fluo-4 and confocal microscopy in beating clusters and in isolated iPSC-CM. Ca^{2+} overload was induced in both preparations by treatment with ouabain (2.5-5 uM or with isoproterenol (1 uM) plus 5.4 mM extracellular Ca^{2+}. Both maneuvers produced a rise in diastolic Ca^{2+} as well as the appearance of oscillatory action potentials, putative delayed afterdepolarizations and triggered activityies in beating clusters. These results indicate that iPS-derived cardiomyocytes provide a useful platform to study the mechanisms of Ca^{2+} overload-induced arrhythmias as well as possible treatments. However, given the spontaneous nature of iPS-derived cardiomyocytes, these cells may more readily recapitulate the effects of Ca^{2+} overload on sinoatrial or latent pacemaker cells.

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Characterization of a Transient Outward K^+ Current in Hips-Derived Cardiomyocytes

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Background: Human induced pluripotent stem cell (hiPS)-cardiomyocytes can be used to create *in vitro* models of genetic disease such as Brugada Syndrome (BrS). Central to the development of BrS is the Ca^{2+}-independent transient outward K^+ current (I_{to}). In this study, we characterized I_{to} in single hiPS -cardiomyocytes and determined its functional role in beating clusters.

Methods: Embryoid bodies (EBs) were made from a hiPS cell line reprogrammed with Oct4, Nanog, Lin28 and Sox2. Whole cell patch clamp was used to record I_{to} in single hiPS cardiomyocytes. Action potential (AP) recordings from spontaneously beating clusters (BCs) were made using sharp microelectrodes. All recordings were done at 36°C.

Results: BCs exhibited spontaneous APs with an average rate of 54.9 ± 30.1 bpm and maximum diastolic potential (MDP) of -65.6 ± 9.3 mV (n=122). A small phase 1 repolarization which could be blocked by 4-AP (1 mM) was observed in 6/149 hiPS BCs suggesting the presence of I_{to}. Interestingly, in single dissociated hiPS cardiomyocytes, patch clamp analysis revealed a robust I_{to} (13.4 ± 1.79 pA/pF at +40 mV, n=14) in the majority of cells studied. Recovery of I_{to} (at −80 mV) showed a fast and slow phase as follows: i) $1=271 \pm 93$ ms and $2= 2697 \pm 103$ (n=8 cells). These observations demonstrate that I_{to} is present but the slow recovery suggests minimal contribution during the course of an action potential. Mathematical modeling of APs from hiPSC-CMs confirmed these observations.

Conclusion: There is a disconnect between the presence of I_{to} in cells and the absence of phase 1 repolarization in BCs. **I**n BCs the depolarized MDP and fast spontaneous AP rate suggests negligible contribution of I_{to} to phase 1 repolarization. Our results point to an important deficiency of hiPSC-CMs in recapitulating the phenotype of adult native myocytes.

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Analysis of Zolpidem-Induced Long Qt Syndrome in Recombinant hERG Channels and Stem Cell Derived Human Cardiomyocytes

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Zolpidem, a short-acting hypnotic drug prescribed to treat insomnia, has been clinically associated with acquired long QT syndrome (acLQTS) and torsade de pointes tachyarrhythmias (TdP). Because acLQTS is most often precipitated by a reduction of hERG/I_{Kr} currents, we have studiedacute hERG block by zolpidem in HEK cells using patch clamp recordings. We found that zolpidem reduced hERG currents with an IC_{50} value of 65.5 ± 4.5 μM (n=3-6). In marked contrast to many other hERG blockers, hERG surface expression was not impaired on long-term drug exposure. To determine whether zolpidem effects in an expression system are relevant to cardiac electrophysiology, we studied human stem cell-derived cardiomyocytes (iCells, CDI, Madison, WI). In human cardiomyocytes, zolpidem prolonged APD_{90} significantly from 321.2 ± 45.5 ms (n = 9) under control conditions to 375.8 ± 55.5 ms (n=9) and 414.1 ± 54.8 ms (n=9) in the presence of 10 μM and 30 μM zolpidem, respectively. Because zolpidem produced TdP in combination with the antiarrhythmic drug amiodarone, we investigated whether both drug effects were additive. When amiodarone was applied separately, APD_{90} prolonged from 287.5 ± 30.8 ms (n=8) under control conditions to 349.6 ± 50.4 ms, and 403.0 ± 59.1 ms (n=8) with 100 nM and 1 μM amiodarone (n=7), respectively. When amiodarone (100nM) and zolpidem were co-administered, APD_{90} was further prolonged from 341.0 ± 45.3 ms (n=8) during amiodarone treatment to 381.3 ± 38.1 ms with 10 μM (n = 8) and 450.3 ± 50.2 ms with 30 μM zolpidem (n = 8). Taken together, our results indicate that acute zolpidem administration blocks hERG channels and induces cardiac action potential changes that are consistent with clinically observed QT prolongation, particularly in patients treated with multiple medications.

Primary Transporters & Exchangers

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Time Resolved FRET in the SR Ca-ATPase

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We have detected structural dynamics of the sarcoplasmic reticulum Ca-ATPase (SERCA) using time-resolved fluorescence spectroscopy. The Ca-ATPase from fast-twitch skeletal muscle (SERCA1a isoform) was labeled with cyan fluorescent protein (CFP) at the N-terminus in the actuator domain (A) and fluorescein isothiocyanate (FITC) at Lys-515 in the nucleotide-binding domain (N). Time-resolved FRET was detected between CFP (donor in A domain) and FITC (acceptor in N domain) for SERCA in ligand-stabilized states, including calcium-free (E2), calcium-bound (E1), and actively-cycling phosphoenzyme (EP). Lifetime fitting and molecular modeling were used to interpret fluorescence decays, thereby identifying a dynamic distribution of structural states within the cytoplasmic headpiece of SERCA